Pergamon

Biochemical Pharmacology, Vol. 48, No. 11, pp. 2057–2063, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00413-7

EFFECT OF 1-(5-ISOQUINOLINESULFONYL)-2-METHYLPIPERAZINE (H-7) ON HSP70 AND HSP28 GENE EXPRESSION AND THERMOTOLERANCE DEVELOPMENT IN HUMAN COLON CARCINOMA CELLS

YONG J. LEE,* CHRISTINE M. BERNS, GEZA ERDOS, MICHAEL J. BORRELLI, CHANG H. AHN† and PETER M. CORRY

Department of Radiation Oncology, William Beaumont Hospital, Royal Oak, MI 48073; and †FDA, Rockville, MD 20857, U.S.A.

(*Received* 2 *June* 1994; *accepted* 4 *August* 1994)

Abstract—The effect of 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7), a potent protein kinase C (PKC) inhibitor, on the development of thermotolerance and expression of heat shock genes (HSP70 and HSP28) was investigated in human colon carcinoma HT-29 cells. After acute heating at 45° for 15 min, cells became resistant to a challenge heat shock. The development of thermotolerance was suppressed by adding H-7 after heat shock. Northern blots show that the levels of HSP70 and HSP28 mRNA increased rapidly and reached maximal values within 6 hr. H-7 suppressed the accumulation of HSP70 and HSP28 mRNA as well as their protein synthesis, and the level of suppression was concentration dependent. However, little effect was observed if the drug was added 1 hr before and during heat shock. These results suggest that PKC is involved in the regulation of heat shock gene expression after acute heat shock.

Key words: H-7; northern blot; western blot; protein kinase C; thermotolerance

Heat shock genes are expressed in response to a wide range of stresses, including heat shock exposure [1], viral infection [2], and treatment with chemical agents [3]. Although the role of HSPs‡ has been a matter of speculation ever since their discovery [1], it is generally believed that the primary function of these proteins is protective, e.g. stabilization and/or solubilization of particular target proteins [4–6]. Heat-induced preferential synthesis of HSPs, particularly HSP70 has been correlated with the induction of transient heat resistance (thermotolerance) [7, 8]. The development of thermotolerance is accompanied, at least partly, by increased synthesis of HSP70, and HSP70 returns to control levels as thermotolerance decays [8]. Moreover, recent studies show that Rat-1 cells, which were transfected with a human HSP70 gene in pSV-hsp70, contained an elevated level of HSP70 and became intrinsically resistant to heat killing [9]. The development of thermotolerance is altered when heat shock gene expression is suppressed [10, 11].

Recently, several researchers reported that quercetin, a bioflavonoid compound, suppresses the expression of heat shock genes and the development of thermotolerance [10, 12, 13]. The biochemical and physiological effects of the drug are rather diverse: inhibition of cell growth by blocking cell progression from the G_1 to S phase [14], inhibition of the lactate transport system and glycolysis process [15], and inhibition of various enzymes including phosphatidylinositol 3-kinase [16], PKA [17] and PKC [18]. We hypothesized that inhibition of PKC is responsible for the suppression of heat shock gene expression and thermotolerance development. To test the hypothesis, we chose potent PKC inhibitors, isoquinolinesulfonamide derivatives. We selected H-7, HA1004, and H-8 among the derivatives. H-7 is known to be the most potent and selective PKC inhibitor among them [19]. The effect of this drug was compared with the effects of HA1004 and H-8. HA1004 is the weakest PKC inhibitor among the derivatives and is used as a control for H-7. H-7 and HA1004 inhibit PKA to almost the same extent. H-8 actively inhibits both PKC and PKA [19]. Our data demonstrated that H-7 and H-8 inhibited HSP70 and HSP28 gene expression as well as thermotolerance development. In contrast, HA1004 did not affect HSP70 gene expression.

MATERIALS AND METHODS

Cell culture and survival determination. Exponentially growing human colon carcinoma HT-29 cells were cultured in McCoy's 5a medium (Cellgro). The medium was supplemented with 26 mM sodium bicarbonate and 10% iron-supplemented calf serum

* Corresponding author: Dr. Yong J. Lee, Department of Radiation Oncology, Research Laboratories, William Beaumont Hospital, 3601 West Thirteen Mile Road, Royal Oak, MI 48073. Tel (810) 551-2568; FAX (810) 551-2443.

‡ Abbreviations: HSPs, heat shock proteins; H-7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine; H-8, *N*-(2-methylaminoethyl)-5-isoquinolinesulfonamide; HA1004, *N* - (2 - guanidinoethyl) - 5 - isoquinolinesulfonamide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IP_3, inositol 1,4,5-triphosphate; DAG, diacylglycerol; PKC, protein kinase C; PKA, protein kinase A; and HSP70, 70 kDa heat shock protein.

(HyClone). T-25 flasks containing cells were kept in a 37° humidified incubator with a mixture of 95% air and 5% CO_2. For survival determination, cells were trypsinized, counted, and plated at appropriate dilutions. After 2–3 weeks of incubation at 37° colonies were stained and counted.

Hyperthermic treatment. T-75 flasks or 35-mm petri dishes containing cells were heated by total immersion in a circulating water bath (Heto) maintained within ±0.05° of the desired temperature.

Drug treatment. Isoquinolinesulfonamide derivatives, H-7 (mol. wt 364.3), H-8 (mol. wt 338.3) and HA1004 (mol. wt 329.8), were obtained from the Sigma Chemical Co. Drug treatment was accomplished by aspirating the normal medium and replacing it with medium containing a drug. The drug treatment was terminated by aspiration and rinsing with Hanks' balanced salt solution (HBSS).

Measurement of PKC activity. Amersham's PKC enzyme assay system was used to determine PKC activity in a variety of samples. Cells from T-25 flasks (approximately 3×10^6 cells) were mixed with 100 μL extraction buffer [50 mM Tris–HCl, pH 7.5, 5 mM EDTA, 10 mM EGTA, 0.3% (w/v) β-mercaptoethanol, 50 μg/mL phenylmethylsulfonyl fluoride (PMSF), 1 μg/mL leupeptin], sonicated, and then centrifuged. Supernatants were saved and mixed with glycerol for storage. Protein content was determined by the method of Bradford [20]. For reaction, 25 μL of the buffer solution (calcium buffer, lipid, peptide buffer, and dithiothreitol buffer from the Amersham assay kit) was added to 25 μL of the sample (50–100 μg protein). The reaction was started by adding 1 μCi [^{32}P]ATP (sp. act. 650 Ci/mmol, ICN) and continued for 15 min at 25°. The reaction was terminated by adding stop reagent. The radioactivity of the control was approximately 590 cpm/μg protein.

Western blot analysis. Cells were lysed with sample buffer (2.4 M glycerol, 0.14 M Tris, pH 6.8, 0.21 M SDS, 0.3 mM bromphenol blue). Lysates from equal amounts of protein (50 μg) were separated by one-dimensional SDS–PAGE. After electrophoresis, the proteins were transferred onto a nitrocellulose membrane and processed for immunoblotting with the HSP70 monoclonal antibody (Amersham). The HSP70 antibody was diluted 1:500. Alkaline phosphatase-conjugated rabbit-antimouse IgG (diluted 1:800) was used to detect the primary antibody.

Northern blot analysis. Total cellular RNA was extracted by the LiCl-urea method of Tushinski *et al.* [21]. For RNA analysis, 30 μg of total RNA was electrophoresed in a 1% agarose-formaldehyde gel [22]. The RNA was blotted from the gels onto nitrocellulose membranes and baked at 80° for 2 hr in a vacuum oven. Membranes were prehybridized at 42° in 50% formamide, 1 × Denhardt's solution, 25 mM KPO_4 (pH 7.4), 5 × SSC (1 × SSC = 150 mM NaCl, 15 mM $Na_3C_6H_5O_7$), and 50 μg/mL denatured and fragmented salmon sperm DNA. Hybridizations were at 42° in prehybridization solution containing 10% dextran sulfate and radiolabeled human inducible HSP70 cDNA probes (R. Morimoto, Northwestern University), human HSP28 cDNA probes (StressGen Biotechnologies Corp.) or human GAPDH cDNA probes (American Type Culture Collection) at a concentration of 1.5×10^6 cpm/mL, 4×10^6 cpm/mL, or 1×10^6 cpm/mL, respectively. Posthybridization, blots were washed twice in 2 × SSC for 15 min at room temperature, washed once in 0.5 × SSC and 0.1% SDS for 30 min at 50°, and washed twice in 0.2 × SSC and 0.1% SDS for 1.5 hr at 50°. Blots were placed into a stainless steel cassette with an intensifying screen and autoradiographed. Blots were stripped to allow hybridization with another probe by washing the membrane with sterile boiling water for 2 min, and then the membrane was prehybridized immediately.

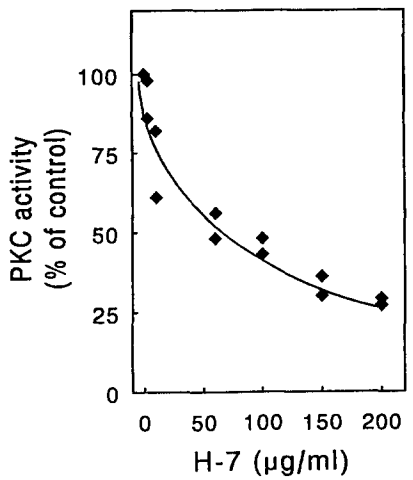

Fig. 1. Inhibition of PKC activity by treatment with various concentrations of H-7 in HT-29 cells. Data from two separate experiments are compiled.

RESULTS

Inhibition of PKC activity by H-7. PKC activity was inhibited by treatment with various concentrations of H-7 (Fig. 1). The drug concentration that inhibited the enzyme activity by 50% (IC_{50}) was 60 μg/mL in human colon carcinoma HT-29 cells. In these experiments, the drug was added to whole cell extracts and the PKC activity was measured in a cell-free system. The IC_{50} values of the drug in the cell-free system and in the *in vivo* system are probably somewhat different.

Effect of pretreatment with H-7 on HSP70 and HSP28 gene expression. Northern blots demonstrated that the levels of HSP70 and HSP28 mRNA increased rapidly and reached maximal values within 6 hr after heat shock at 45° for 15 min (Fig. 2). H-7 (60 μg/mL) did not suppress the accumulation of HSP70 and HSP28 mRNA when the drug was added 1 hr before and left in during heat shock (Fig. 2). Control hybridization with a probe for the "household" enzyme GAPDH showed that pretreatment with H-7 did not induce any major changes in the GAPDH mRNA level. Data from western blot analysis demonstrated that heat shock increased the levels of constitutive and inducible HSP70 (Fig. 3).

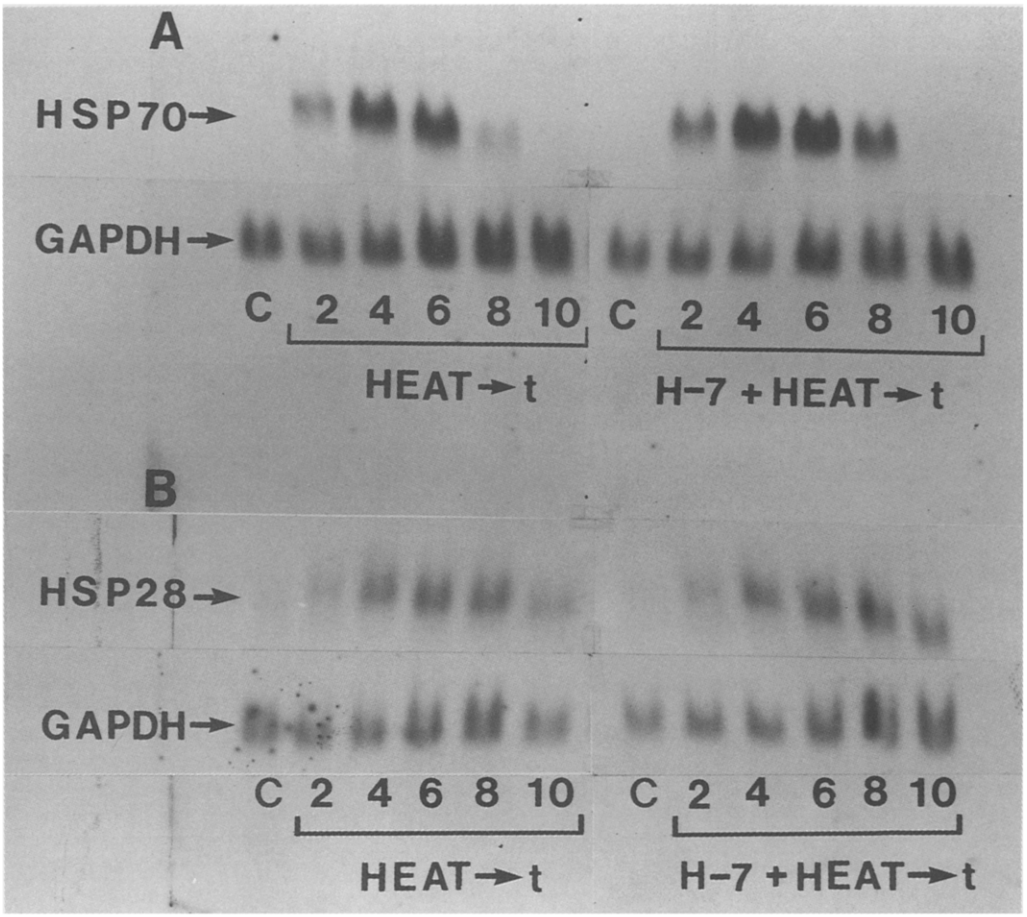

Fig. 2. Effect of pretreatment with H-7 on accumulation of inducible HSP70 (A) and HSP28 (B) mRNA after heat shock. **HEAT→t:** Cells were heated at 45° for 15 min, and incubated at 37° for the intervals (2, 4, 6, 8, 10 hr) indicated at the bottom of each lane. **H-7 + HEAT→t**: Cells were exposed to H-7 (60 μg/mL) for 1 hr before and during heat shock at 45° for 15 min. Immediately after heat shock, cells were rinsed twice with HBSS and then incubated at 37° for the intervals (2, 4, 6, 8, 10 hr) indicated at the bottom of each lane. **C**: Unheated control cells. **GAPDH**: Differences in RNA loading were examined by rehybridizing the nitrocellulose membranes with the housekeeping gene, GAPDH probe.

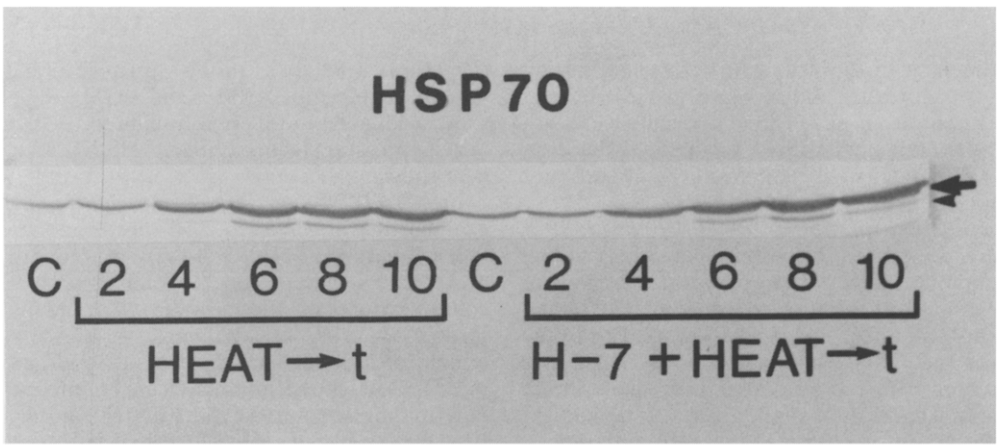

Fig. 3. Effect of pretreatment with H-7 on levels of HSP70 after heat shock. **HEAT→t**: Cells were heated at 45° for 15 min, and incubated at 37° for the intervals (2, 4, 6, 8, 10 hr) indicated at the bottom of each lane. **H-7 + HEAT→t**: Cells were exposed to H-7 (60 μg/mL) for 1 hr before and during heat shock at 45° for 15 min. Immediately after heat shock, cells were rinsed twice with HBSS and then incubated at 37° for the intervals (2, 4, 6, 8, 10 hr) indicated at the bottom of each lane. **C**: Unheated control cells. Constitutive HSP70 and inducible HSP70 are identified by arrow and arrowhead, respectively.

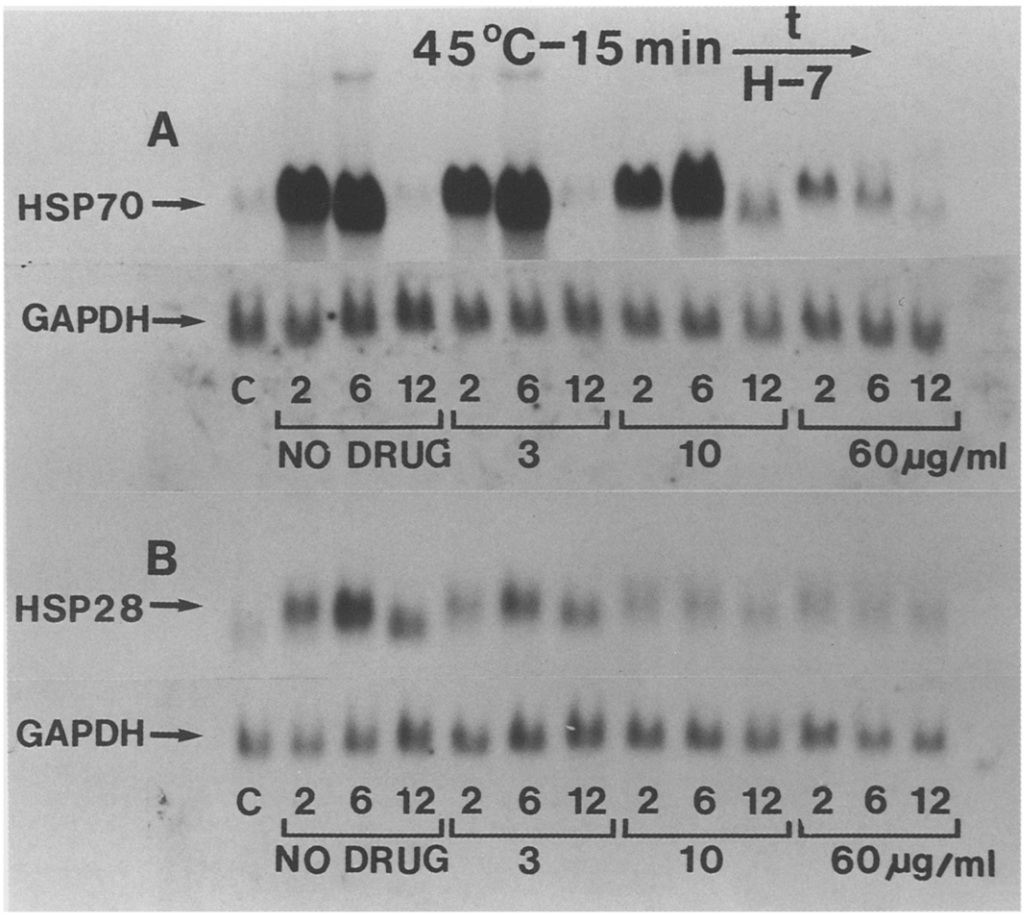

Fig. 4. Effect of treatment with H-7 on accumulation of inducible HSP70 (A) and HSP28 (B) mRNA after heat shock. Cells were heated at 45° for 15 min and incubated at 37° for the intervals (2, 6, 12 hr) indicated at the bottom of each lane. During the incubation period, cells were treated without H-7 (NO DRUG) or with the drug (3, 10, 60 μg/mL). **C**: Unheated untreated control cells. **GAPDH**: Differences in RNA loading were examined by rehybridizing the nitrocellulose membranes with the housekeeping gene, GAPDH probe.

Pretreatment with the drug 1 hr before and during heating did not alter the increase in HSP70 level after heat shock (Fig. 3). Similar results were still observed even if higher concentrations of the drug (100–300 μg/mL) were administered (data not shown).

Effect of treatment with H-7 on HSP70 and HSP28 gene expression after heat shock. Data presented in Fig. 4 show the effect of various concentrations of H-7 treatment on the accumulation of HSP70 and HSP28 mRNA after heat shock. Suppression of the HSP gene encoding mRNA was observed when cells were treated with the drug after heat shock at 45° for 15 min. The level of suppression was dependent upon the drug concentrations. H-7 (60 μg/mL) markedly suppressed the levels of both HSP gene encoding mRNAs, whereas the drug did not affect the GAPDH mRNA level. Data from western blot analysis clearly demonstrated that treatment with H-7 (60 μg/mL; 165 μM), as well as H-8 (60 μg/mL; 177 μM), after heat shock significantly suppressed the level of HSP70 (Fig. 5). In contrast, HA1004 (60 μg/mL; 182 μM), an H-7 analogue that is a potent inhibitor of cAMP and cGMP-dependent protein kinases and a weak inhibitor of PKC, did not suppress an increase in the HSP70 level after heat shock.

Effect of H-7 on thermotolerance development. To examine the effect of H-7 on the development of thermotolerance, HT-29 cells were heated for 15 min at 45° and incubated at 37° for 6 hr with various concentrations of the drug (3–60 μg/mL). After incubation, cells were challenged at 45° for various lengths of time (1–4 hr). Effects of the drug on the development of thermotolerance were measured by determining the survival level after the challenge heat treatment (Fig. 6). The initial heat treatment killed 10% of the cells (data not shown) and made the surviving cells thermotolerant to a second heat treatment. Thermotolerance reached its maximum 6 hr after the initial heat treatment, maintained that level for 48 hr, and then gradually decayed (data not shown). Treatment with the drug without initial heat shock altered heat sensitivity very little (data not

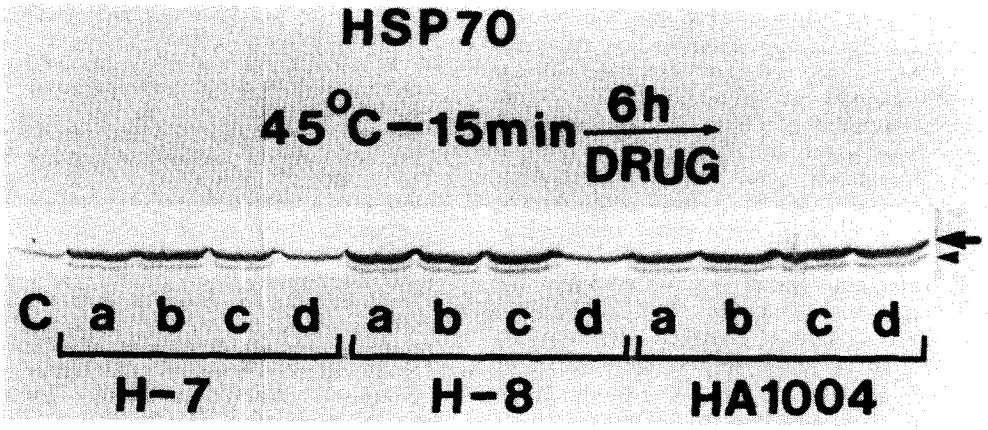

Fig. 5. Effect of treatment with H-7, H-8, or HA1004 on levels of HSP70 after heat shock. Cells were heated at 45° for 15 min and incubated at 37° for 6 hr with various concentrations (**a**: no drug, **b**: 3, **c**: 10, **d**: 60 μg/mL) of H-7, H-8, or HA1004. C: Unheated untreated control cells. Constitutive HSP70 and inducible HSP70 are identified by arrow and arrowhead, respectively.

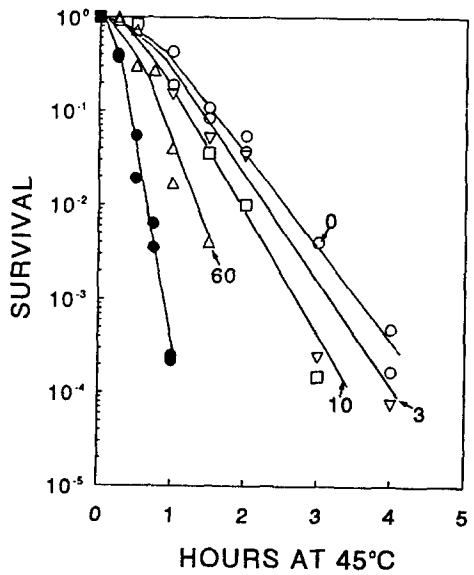

Fig. 6. Effect of H-7 (3–60 μg/mL) on the development of thermotolerance. Cells were first heated at 45° for 15 min, incubated at 37° for 6 hr with the drug (○: 0, ▽: 3, □: 10, △: 60 μg/mL), and then challenged at 45°. ● = survival curve of control cells heated at 45°. The data are a compilation of two separate experiments.

shown). For example, pretreatment with 60 μg/mL H-7 reduced survival from 3.5×10^{-3} to 8×10^{-4} after 45 min at 45°. The drug-induced heat sensitization effect was normalized. H-7 (3–60 μg/mL) treatment alone caused minimum cytotoxicity. Plating efficiency was 71, 73, 67, or 62% for 0, 3, 10, or 60 μg/mL H-7 treatment for 6 hr, respectively. Survival was normalized for drug cytotoxicity.

DISCUSSION

Several conclusions can be drawn upon consideration of the data presented. Heat-induced thermotolerance was suppressed effectively by treatment with H-7 (60 μg/mL), a potent PKC inhibitor, after initial heat shock in HT-29 cells (Fig. 6). The drug treatment also suppressed an increase in HSP28 and HSP70 mRNA levels as well as their protein levels after heat shock (Figs. 4 and 5). Similar results were observed when H-8, another strong PKC inhibitor, was added after initial heat shock (Fig. 5). In contrast, HA1004, which is a strong PKA inhibitor but a weak PKC inhibitor, did not alter HSP28 and HSP70 gene expression (Fig. 5). These results imply that PKC and not PKA is involved in HSP28 and HSP70 gene regulation. They are also consistent with previous reports that demonstrate inhibition of heat shock gene expression and thermotolerance development by treatment with staurosporine, another inhibitor of PKC [11].

Recent studies show that heat shock increases cytosolic PKC activity [23]. Heat shock activates phosphatidyl inositol-4,5-bisphosphate phosphodiesterase which is involved in the production of IP_3 and DAG [24]. Heat shock also increases intracellular free Ca^{2+} [25]. The increased DAG and free intracellular Ca^{2+} levels may be responsible for heat-induced activation of PKC, particularly the calcium-dependent PKC family (α, βI, βII, and γ) [23]. When the intracellular concentration of free Ca^{2+} rises, PKC translocates to the plasma membrane where it is activated in the presence of DAG [26–29]. The maximum activation of PKC occurred within 3 hr after heating at 45° for 15 min (unpublished data). These observations may explain why pretreatment with the drug 1 hr before and during initial heat shock did not alter HSP28 and HSP70 gene expression (Figs. 2 and 3). These results implicate PKC as a modulator of heat shock gene expression following heat shock. Similar results have been observed in X-ray irradiated human A549 lung adenocarcinoma cells [30]. The maximum induction of PKC ε by 150 cGy of ionizing radiation requires 1 hr post-incubation. However, Hallahan *et al.* [31] reported that PKC-dependent genes respond rapidly

within minutes following irradiation in human HL-60 leukemic cells. It still remains to be determined whether PKC is involved in the activation of the HSP genes. The fact that the addition of H-7 before and during heat shock had little effect on heat shock gene expression argues that it may not.

Cell fractionation and immunocytochemical studies have shown that PKC exists in the cytosol, membranes, and associates with intermediate filaments [32]. The distribution of PKC changes with its activation. The heat-activated PKC may redistribute into the nucleus. This topographical rearrangement of PKC is observed when cells are treated with stresses such as phorbol 12-myristate 13-acetate (PMA) [33]. Nuclear localization of the enzyme may play a role in gene expression [33]. PKC has also been shown to affect NF-κB relocalization by dissociating the NF-κB-IκB complex. The phosphorylation of IκB by PKC results in the dissociation of the NF-κB-IκB complex [34]. Activated NF-κB is then able to enter the nucleus and subsequently activate heat shock gene transcription.

At the present time, only speculations can be made concerning the mechanism of PKC involvement in heat shock gene transcription. Heat-activated PKC could be involved in the heat shock gene transcription through posttranslational modification of transcription factors such as heat shock transcription factor. Heat shock gene promoters contain various regulatory elements that serve as the binding sites for inducible and basal transcription factors [35–37]. These transcription factors interact with each other to regulate transcription of heat shock genes. The transcription factors can be activated or inactivated through posttranslational modification of proteins, such as phosphorylation, dephosphorylation, or glycosylation [38]. The modification of proteins can effectively integrate information carried by multiple signal transduction pathways in the absence of new protein synthesis [39]. The signal transduction pathways frequently employ kinases and phosphatases to regulate transcription factor activity [38]. However, most of these enzymes, that are involved in the signal transduction pathways for the regulation of heat shock gene transcription, have not been identified. In this paper, we present the possible involvement of PKC in heat shock gene regulation as a framework for future experiments. However, other possibilities, such as activation of different kinases by heat shock, should be considered [40, 41].

Acknowledgements—This research was supported by NCI Grants CA48000, CA44550, and CA53114, and the William Beaumont Hospital Research Institute Grant 93-09.

REFERENCES

1. Tissieres A, Mitchell HK and Tracy U, Protein synthesis in salivary glands of *Drosophila melanogaster*. Relation to chromosome puffs. *J Mol Biol* **84**: 389–398, 1974.
2. Peluso RW, Lamb RA and Choppin PW, Polypeptide synthesis in simian virus 5-infected cells. *J Virol* **23**: 177–187, 1977.
3. Li GC, Induction of thermotolerance and enhanced heat shock protein synthesis in Chinese hamster fibroblasts by sodium arsenite and by ethanol. *J Cell Physiol* **115**: 116–122, 1983.
4. Pelham HRB, Speculations on the functions of the major heat shock and glucose-regulated proteins. *Cell* **46**: 959–961, 1986.
5. Jakob U, Gaestel M, Engel K and Buchner J, Small heat shock proteins and molecular chaperones. *J Biol Chem* **268**: 1517–1520, 1993.
6. Kim D, Lee YJ and Corry PM, Employment of a turbidimetric assay system to study the role of HSP70 in heat-induced protein aggregation. *J Therm Biol* **18**: 165–175, 1993.
7. Li GC and Mak JY, Induction of heat shock protein synthesis in murine tumors during the development of thermotolerance. *Cancer Res* **45**: 3816–3824, 1985.
8. Li GC, Elevated levels of 70,000 dalton heat shock protein in transiently thermotolerant Chinese hamster fibroblasts and their stable heat resistant variants. *Int J Radiat Oncol Biol Phys* **11**: 165–177, 1985.
9. Li GC, Li L, Liu Y-K, Mak JY, Chen L and Lee WMF, Thermal response of rat fibroblasts stably transfected with the human 70-kDa heat shock protein-encoding gene. *Proc Natl Acad Sci USA* **88**: 1681–1685, 1991.
10. Lee YJ, Curetty L, Hou Z, Kim SH, Kim JH and Corry PM, Effect of pH on quercetin-induced suppression of heat shock gene expression and thermotolerance development in HT-29 cells. *Biochem Biophys Res Commun* **186**: 1121–1128, 1992.
11. Kim SH, Kim JH, Erdos G and Lee YJ, Effect of staurosporine on suppression of heat shock gene expression and thermotolerance development in HT-29 cells. *Biochem Biophys Res Commun* **193**: 759–763, 1993.
12. Hosokawa N, Hirayoshi K, Nakai A, Hosokawa Y, Marui N, Yoshida M, Sakai T, Nishino H, Aoike A, Kawai K and Nagata K, Flavonoids inhibit the expression of heat shock proteins. *Cell Struct Funct* **15**: 393–401, 1990.
13. Koishi M, Hosokawa N, Sato M, Nakai A, Hirayoshi K, Hiraoka M, Abe M and Nagata K, Quercetin, an inhibitor of heat shock protein synthesis, inhibits the acquisition of thermotolerance in a human colon carcinoma cell line. *Jpn J Cancer Res* **83**: 1216–1222, 1992.
14. Yoshida M, Sakai T, Hosokawa N, Marui N, Matsumoto K, Fujioka A, Nishino H and Aoike A, The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. *FEBS Lett* **260**: 10–13, 1990.
15. Belt JA, Thomas JA, Buchsbaum RN and Racker E, Inhibition of lactate transport and glycolysis in Ehrlich ascites tumor cells by bioflavonoids. *Biochemistry* **18**: 3506–3511, 1979.
16. Matter WF, Brown RF and Vlahos CJ, The inhibition of phosphatidylinositol 3-kinase by quercetin and analogs. *Biochem Biophys Res Commun* **186**: 624–631, 1992.
17. Graziani Y, Chayoth R, Karny N, Feldman B and Levy J, Regulation of protein kinases activity by quercetin in Ehrlich ascites tumor cells. *Biochim Biophys Acta* **714**: 415–421, 1981.
18. Gschwendt M, Horn F, Kittstein W and Marks F, Inhibition of the calcium- and phospholipid-dependent protein kinase activity from mouse brain cytosol by quercetin. *Biochem Biophys Res Commun* **117**: 444–447, 1983.
19. Hidaka H, Inagaki M, Kawamoto S and Sasaki Y, Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry* **23**: 5036–5041, 1984.
20. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing

the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
21. Tushinski R, Sussman P, Yu L and Bancroft F, Pregrowth hormone messenger RNA: Glucocorticoid induction and identification in rat pituitary cells. *Proc Natl Acad Sci USA* **74**: 2357–2361, 1977.
22. Lehrach H, Diamond L, Wozney J and Boedtker H, RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry* **16**: 4743–4751, 1977.
23. Wooten MW, Alterations in protein kinase C type III-α during heat shock of rat embryo fibroblasts. *Exp Cell Res* **193**: 274–278, 1991.
24. Calderwood SK, Stevenson MA and Hahn GM, Heat stress stimulates inositol trisphosphate release and phosphorylation of phosphoinositides in CHO and BalbC3T3 cells. *J Cell Physiol* **130**: 369–376, 1987.
25. Vidair CA, Wang Z and Dewey WC, Noninvolvement of the heat-induced increase in the concentration of intracellular free Ca^{2+} in killing by heat and induction of thermotolerance. *Radiat Res* **124**: 156–164, 1990.
26. Boni LT and Rando RR, The nature of protein kinase C activation by physically defined phospholipid vesicles and diacylglycerols. *J Biol Chem* **260**: 10819–10825, 1985.
27. Wolf M, LeVine H III, May WS Jr, Cuatrecasas P and Sahyoun N, A model for intracellular translocation of protein kinase C involving synergism between Ca^{2+} and phorbol esters. *Nature* **317**: 546–549, 1985.
28. Gopalakrishna R, Barsky SH, Thomas TP and Anderson WB, Factors influencing chelator-stable, detergent-extractable, phorbol diester-induced membrane association of protein kinase C. Differences between Ca^{2+}-induced and phorbol ester-stabilized membrane bindings of protein kinase C. *J Biol Chem* **261**: 16438–16445, 1986.
29. Mosior M and McLaughin S, Peptides that mimic the pseudosubstrate region of protein kinase C bind to acidic lipids in membranes. *Biophys J* **60**: 149–159, 1991.
30. Kim CY, Giaccia AJ, Strulovici B and Brown JM, Differential expression of protein kinase C ε protein in lung cancer cell lines by ionising radiation. *Br J Cancer* **66**: 844–849, 1992.
31. Hallahan DE, Virudachalam S, Sherman ML, Huberman E, Kufe DW and Weichselbaum RR, Tumor necrosis factor gene expression is mediated by protein kinase C following activation by ionizing radiation. *Cancer Res* **51**: 4565–4569, 1991.
32. Murti KG, Kaur K and Goorha RM, Protein kinase C associates with intermediate filaments and stress fibers. *Exp Cell Res* **202**: 36–44, 1992.
33. Nishizuka Y, Studies and perspectives of protein kinase C. *Science* **233**: 305–312, 1986.
34. Ghosh S and Baltimore D, Activation *in vitro* of NF-κB by phosphorylation of its inhibitor IκB. *Nature* **344**: 678–682, 1990.
35. Chang N-T, Huang LE and Liu AY-C, Okadaic acid markedly potentiates the heat-induced hsp 70 promoter activity. *J Biol Chem* **268**: 1436–1439, 1993.
36. Lis J and Wu C, Protein traffic on the heat shock promoter: Parking, stalling, and trucking along. *Cell* **74**: 1–4, 1993.
37. Tsukiyama T, Becker PB and Wu C, ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature* **367**: 525–532, 1994.
38. Jackson SP and Tjian R, O-Glycosylation of eukaryotic transcription factors: Implications for mechanisms of transcriptional regulation. *Cell* **55**: 125–133, 1988.
39. Zimarino V and Wu C, Induction of sequence-specific binding of *Drosophila* heat shock activator protein without protein synthesis. *Nature* **327**: 727–730, 1987.
40. Kyriakis JM, Banerjee P, Nikolakaki E, Dai T, Rubie EA, Ahmad MF, Avruch J and Woodgett JR, The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* **369**: 156–160, 1994.
41. Engelberg D, Zandi E, Parker CS and Karin M, The yeast and mammalian Ras pathways control transcription of heat shock genes independently of heat shock transcription factor. *Mol Cell Biol* **14**: 4929–4937, 1994.